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Enzymatic Synthesis of Cocarboxylase

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This report is concerned with the synthesis of cocarboxylase from thiamine hydrochloride and acetyl-phosphate by *Lactobacillus delbrückii*. This bacillus was cultivated in 100 ml. of the malt solution for 48 hrs., centrifuged and dried by addition of cold acetone. The dried material was used at once. Acetyl-phosphate was prepared from isopropenyl acetate and H_3PO_4. For elucidation of the formation of cocarboxylase, the following experiments had been carried out: (1) measurements of the Δ7P hydrolysed by 1-N HCl. (2) Partitional paperchromatography (PPC). (3) Measurements of CO_2 decarboxylated by the carboxylase from pyruvate by the Warburg apparatus. The acetone powder of Lact. delb., 16.6 mg. of acetyl-P, 3.3 mg. of V.B_1 and 0.3 mg. of $MgCl_2.6H_2O$ were mixed with 1.5 ml. of acetate buffer, pH 6.2. This mixture was placed at 37°C for 90 min. The amounts of inorganic phosphorus were decreased after incubation and a half amounts of acetyl-P added was decomposed. But the amounts of organic phosphorus compounds (especially Δ7P) were increased. Therefore it might be supposed that the synthesis of cocarboxylase occured. By the PPC developed with the solvent of *n*-propanol, water and 1 M acetate buffer of pH 5.0 (7 : 2 : 1), the spot of V.B_1 and the faint spots of TMP and cocarboxylase were recognized. In Warburg's apparatus, the evolution of CO_2 by the active enzyme was greater than that by the heat-treated enzyme system. Mg^{++} activated the evolution of CO_2. V.B_1 was more effective than TMP. The acetone powder of Lact. delb., contained 500γ of inorganic phosphorus and 1800γ of organic phosphorus compounds. It shows therefore that the high energy phosphorus compounds were contained in Lact. delb. already. In fact, even if acetyl-P was not added, the formation of CO_2 was recongnized to a small extent but it is increased greatly by the addition of acetyl-P. If the active dried Lact. delb. was dialysed for 48 hrs. in a refrigerator, all activity was lost. But if the boiled Lact. delb. was added to the inactive dialysed enzyme, its activity was recovered. It seemed that some activating substance may exist in the dialysate.

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